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Influence of encapsulated functional lipids on crystal structure and chemical stability in solid lipid nanoparticles: Towards bioactive-based design of delivery systems

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ABSTRACT

We investigated the influence of physicochemical properties of encapsulated functional lipids – vitamin A, β-carotene and ω-3 fish oil – on the structural arrangement of solid lipid nanoparticles (SLN). The relationship between the crystal structure and chemical stability of the incorporated bioactive lipids was evaluated with different emulsifier compositions of a saponin-rich, food-grade Quillaja extract alone or combined with high-melting or low-melting lecithins. The major factors influencing the structural arrangement and chemical stability of functional lipids in solid lipid dispersions were their solubility in the aqueous phase and their crystallization temperature in relation to that of the carrier lipid. The results showed that the stabilization of the α-subcell crystals in the lattice of the carrier lipid is a key parameter for forming stable solid lipid dispersions. This study contributes to a better understanding of SLN as a function of the bioactive lipid.

1. Introduction

Fortification of foods with β-carotene, vitamin A and ω-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA, C22:6 ω-3) and eicosapentaenoic acid (EPA, C20:5 ω-3) is of high interest due to the increasing nutritional awareness in industrialized countries, as well as prevention of malnutrition in developing countries. The major drawbacks for food fortification with functional lipids are their high susceptibility to oxidative breakdown and their poor solubility in aqueous foods (McClements & Decker, 2000; McClements, Decker, & Weiss, 2007; Weiss et al., 2008). Therefore, the application of suitable carrier systems is necessary in order to improve dispersibility and to allow sufficient protection against oxygen and pro-oxidants, when dissolved in the aqueous food matrix.

Possible types of carrier systems for functional lipids are solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC).

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The composition of SLN-dispersions is similar to oil-in-water emulsions but with a fully crystallized lipid phase, whereas in the NLC the bioactive-containing oil is dispersed in a carrier lipid that is solid at room and body temperature and forms a partially crystallized lipid matrix (Müller, Radtke, & Wissing, 2002). The oxidative stability of functional compounds entrapped into solid matrices may be improved since the diffusion towards the aqueous phase is limited, compared to liquid emulsions (McClements et al., 2007). However, the design of SLN and NLC for the encapsulation of functional lipids brings along some challenges. First, solid lipid matrices have a tendency to recrystallize during storage which can lead to physical and chemical instability. Second, crystallization processes during production are complex and strongly dependent on the particular lipid composition and the production conditions. Different locations of the functional compound in the lipid particle with different release profiles are possible (Müller, Mäder, & Gohla, 2000; Müller et al., 2002).

Surfactants have been shown to be a key factor for the structural arrangement and stability of SLN and NLC (Helgason, Awad, Kristbergsson, Decker, et al., 2009; Salminen, Aulbach, Leuenberger, Tedeschi, & Weiss, 2014; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013). Still, more research is needed in order to understand the relationship between composition of lipid nanoparticles and their crystal structure and stabilization. In previous studies, high-melting lecithins in combination with bile salts as co-surfactants have been shown to be suitable surfactants for SLN production with respect to polymorphic, physical and oxidative stability (Helgason, Awad, Kristbergsson, Decker, et al., 2009; Salminen et al., 2013). However, the application of bile salts in foods is limited due to their bitter taste (Menguy & Peissner, 1960) and high price. Therefore, bile salts need to be replaced by surfactants that comply with the requirements for food applications. Since saponins have structural similarities to bile salts, they may be suitable to replace taurodeoxycholate as a surfactant in SLN and NLC production (Salminen, Aulbach, et al., 2014). Saponins are surface-active glycosides of high molecular weight which are composed of hydrophilic sugar moieties and a hydrophobic triterpene or steroid aglycon (FAO-WHO, 2005; San Martín & Briones, 1999). The extract derived from the bark of the Chilean tree *Quillaja saponaria* is rich in saponins and food approved for water-based, non-alcoholic beverages in the European Union with the number E 999 (FAO-WHO, 2005).

We hypothesized that the chemical structure of the encapsulated functional lipids influences the structural arrangement of lipid particles (SLN and NLC) and thus affects their susceptibility to oxidation. In addition, Quillaja extract has shown potential for good structuring and stabilizing abilities for loaded solid lipid particles due to its structural similarities to bile salts (Salminen, Aulbach, et al., 2014). To test our hypothesis, β-carotene, vitamin A (acetate), and ω-3 fish oil were encapsulated into solid lipid particles stabilized with Quillaja extract, alone or combined with high-melting lecithin or low-melting lecithin. The physical and chemical stability was observed over 51 days of storage. These functional lipids were chosen due to their different physical properties: β-carotene is highly hydrophobic and has a melting point at ~180 °C. In contrast, vitamin A exhibits moderate hydrophobicity with a melting point at ~60 °C, whereas ω-3 fish oil is hydrophobic with a melting point <0 °C.

2. Materials and methods

2.1. Materials

Crystalline β-carotene, crystalline vitamin A acetate (2.8 MIU/g), and MEG-3™ '30' n-3 food oil (a refined fish oil rich in ω-3 PUFAs) were provided by DSM Nutritional Products Ltd. (Basel, Switzerland). The fish oil contained ⩾9% EPA and ⩾12.5% DHA with a total ω-3 PUFA content of ⩾30%. Tristearin Dynasan 118 containing ⩾97% stearic acid was donated by Cremer Oleo GmbH & Co. KG (Witten, Germany). The soy lecithins Phospholipon 80H and Lipoid S 75 were a gift from Lipoid AG (Ludwigshafen, Germany). High-melting lecithin Phospholipon 80H contained ⩾70% hydrogenated phosphatidylcholine and ⩽6% hydrogenated lysophosphatidylcholine. Low-melting lecithin Lipoid S 75 contained ⩾68% lysophosphatidylcholine and ⩾7% phosphatidylethanolamine and linoleic acid as the main fatty acid. *Quillaja saponaria* wood extract (Andean QDP Ultra Organic), a product of Desert King International (San Diego, CA, USA), was purchased from PERA GmbH (Springe-Eldagsen, Germany). The Quillaja extract had a saponin content of 62.5% and 3.38% citric acid as an additive. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4-methylphenol (BHT, purity ⩾99.0%), 2,2′-azo bis(2-methylpropionamidine)dihydrochloride (AAPH, purity 97%), fluorescein sodium salt, (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, purity >98%), sodium phosphate monobasic (purity ⩾99.0%), sodium phosphate dibasic (purity ⩾99.0%), ammonium thiocyanate (purity ⩾99.0%), barium chloride dihydrate (purity ⩾99.0%), iron sulfate heptahydrate (purity ⩾99.0%), cumene hydroperoxide (purity 80%), propionaldehyde (purity ⩾97%) and hexanal (purity 98%) were obtained from Sigma Aldrich Co. (Steinheim, Germany). Glacial acetic acid (purity ⩾99.8%), hydrochloric acid (2 N ± 0.2%), isooctane (purity ⩾99.5%), 2-propanol (purity ⩾99.8%), potassium iodide (purity ⩾99.5%) and sodium azide (purity ⩾99%) as well as HPLC grade solvents, methanol (purity ⩾99.9%), ethanol (purity ⩾99.9%) and n-hexane (⩾98%), were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). 1–Butanol (purity ⩾99.5%) was purchased from AppliChem GmbH (Darmstadt, Germany). Chloroform (purity ⩾99.8%) was obtained from Merck KGaA (Darmstadt, Germany). Potato starch (analytical grade) and sodium thiosulphate solution (0.1 N ± 0.2%) were purchased from VWR International GmbH (Darmstadt, Germany). All materials were used without further purification. Double distilled, deionized water was used throughout the study.

2.2. Solution preparation

A sodium phosphate buffer (10 mM, pH 7) was prepared by dissolving 4.2 mM sodium phosphate monobasic and 5.8 mM sodium phosphate dibasic in double distilled water. In order to prevent bacterial growth, 0.02% (w/v) sodium azide was added. Surfactant solutions (3% w/w) were prepared in sodium phosphate buffer (10 mM, pH 7) in the following combinations: (i) 2% Quillaja extract + 1% Phospholipon 80H (Quillaja-80H), (ii) 2% Quillaja extract + 1% Lipoid S 75 (Quillaja-S75), and (iii) 3% Quillaja extract (Quillaja). The surfactant solutions were tempered in a water bath at 90 °C for 30 min.

2.3. Antioxidant potential of surfactant solutions

2.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH)-test

The antioxidant potential of the surfactant solutions was estimated by the DPPH radical scavenging capacity assay (Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, & Saura-Calixto, 2000). Surfactant solutions were prepared as described in Section 2.2 but without addition of sodium azide to the buffer. BHT (0.05–0.5% w/v) in methanol was used as a standard. The antioxidant concentration, which caused a reduction of 50% of the initial DPPH was calculated from the linear regression and termed as the EC_{50} concentration. Consequently, a small EC_{50}-value indicates a high antioxidant potential.